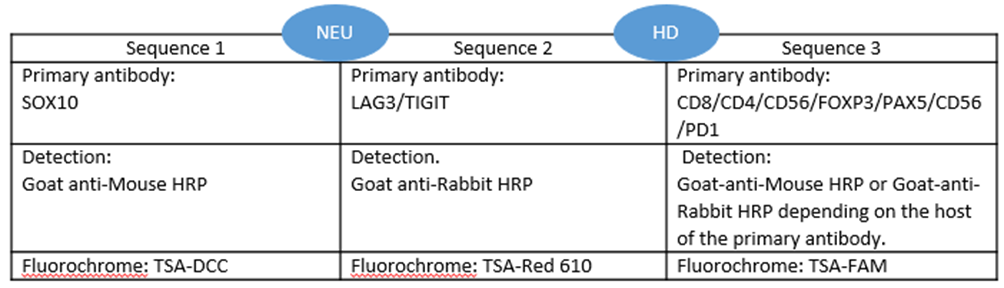
**Supplementary Table S1**. Twenty genes added to the NanoString nCounter PanCancer IO 360 Gene Expression Panel.

|  |  |
| --- | --- |
| **Gene** | **Accession number** |
| AMBRA1 | NM\_017749.2 |
| BAK1 | NM\_001188.2 |
| BRAF | NM\_004333.3 |
| CREM | NM\_181571.2 |
| CYP4A11 | NM\_000778.4 |
| GADD45 | NM\_001924.2 |
| MAP2K1/MEK1 | NM\_002755.2 |
| MAP2K2/MEK2 | NM\_030662.3 |
| MAPK1/erk2 | NM\_138957.2 |
| MAPK3/erk1 | NM\_001040056.1 |
| MCM7 | NM\_182776.1 |
| MDM2 | NM\_001145337.1 |
| MITF | NM\_000248.3 |
| MMP14 | NM\_004995.2 |
| MST1R | NM\_002447.1 |
| PRAME | NM\_006115.4 |
| P48 | NM\_178161.2 |
| FAK1/PTK2 | NM\_005607.3 |
| TANK | NM\_004180.2 |
| TERT | NM\_198253.1 |

**Supplementary Table S2**. Process of sequentual multiplex immunofluorescence



**Supplemtary Table S3**. Assays performed to test for cross reactivity.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Neutralization control | SOX10 | Goat anti-Mouse HRP | NEU |  | Fluorochrome:  TSA-Red 610 |
| HD control | SOX10 | Goat anti-Mouse HRP | HD | Goat anti-mouse HRP | Fluorochrome:  TSA-FAM |
| HD control | TIGIT | Goat anti-Rabbit HRP | HD | Goat anti-Rabbit HRP | Fluorochrome:  TSA-FAM |
| HD control | LAG3 | Goat anti-Rabbit HRP | HD | Goat anti-Rabbit HRP | Fluorochrome:  TSA-FAM |

**Supplementary Table S4.** The 91 differentially expressed genes between brisk TILs and absent TILs (fold change >2, q<0.05)

| **Gene\_Symbol** | **p-value** | **q-value** | **Difference (log2 FC)** |
| --- | --- | --- | --- |
| CXCL9 | 1.14E-12 | 1.25E-11 | 2.82 |
| CXCL10 | 2.18E-10 | 1.45E-09 | 2.41 |
| IDO1 | 6.77E-15 | 1.62E-13 | 1.93 |
| CXCL13 | 9.32E-09 | 4.62E-08 | 1.92 |
| CXCL11 | 1.37E-10 | 9.92E-10 | 1.92 |
| CD27 | 6.06E-15 | 1.50E-13 | 1.81 |
| SH2D1A | 8.29E-17 | 5.08E-15 | 1.75 |
| CD79A | 9.94E-08 | 4.22E-07 | 1.73 |
| GNLY | 5.33E-13 | 6.49E-12 | 1.73 |
| NKG7 | 1.65E-12 | 1.74E-11 | 1.72 |
| KLRK1 | 2.46E-15 | 6.89E-14 | 1.71 |
| GZMK | 1.07E-14 | 2.47E-13 | 1.66 |
| LCK | 2.21E-17 | 3.16E-15 | 1.63 |
| SPIB | 2.58E-15 | 6.94E-14 | 1.62 |
| LAG3 | 8.23E-14 | 1.40E-12 | 1.57 |
| TIGIT | 7.10E-16 | 2.41E-14 | 1.56 |
| CD8A | 2.59E-14 | 5.21E-13 | 1.56 |
| CD3D | 3.12E-16 | 1.34E-14 | 1.55 |
| CD6 | 2.23E-14 | 4.80E-13 | 1.55 |
| CD3E | 6.91E-17 | 5.08E-15 | 1.54 |
| IL7R | 1.73E-15 | 5.08E-14 | 1.54 |
| TRAT1 | 5.26E-16 | 2.00E-14 | 1.53 |
| CD96 | 1.53E-16 | 7.05E-15 | 1.52 |
| ITGAL | 8.67E-17 | 5.08E-15 | 1.51 |
| CD247 | 1.56E-15 | 4.84E-14 | 1.5 |
| CCL5 | 2.57E-14 | 5.21E-13 | 1.5 |
| IL2RB | 4.09E-18 | 2.01E-15 | 1.5 |
| SELL | 3.45E-13 | 4.36E-12 | 1.49 |
| CTLA4 | 1.58E-15 | 4.84E-14 | 1.47 |
| CTSW | 7.97E-14 | 1.39E-12 | 1.46 |
| MS4A1 | 1.17E-11 | 1.04E-10 | 1.46 |
| LTB | 1.70E-13 | 2.39E-12 | 1.45 |
| SLAMF7 | 5.64E-13 | 6.74E-12 | 1.44 |
| IL2RG | 9.35E-18 | 2.01E-15 | 1.44 |
| ZAP70 | 1.39E-16 | 7.05E-15 | 1.43 |
| KLRD1 | 1.12E-12 | 1.25E-11 | 1.43 |
| LYZ | 4.96E-14 | 9.14E-13 | 1.41 |
| TCL1A | 1.73E-08 | 8.39E-08 | 1.4 |
| CCL19 | 2.25E-12 | 2.23E-11 | 1.39 |
| MMP9 | 1.14E-09 | 6.29E-09 | 1.38 |
| CCR4 | 1.74E-12 | 1.81E-11 | 1.38 |
| CD48 | 4.45E-17 | 4.78E-15 | 1.37 |
| CD7 | 9.95E-14 | 1.57E-12 | 1.34 |
| FOXP3 | 7.28E-18 | 2.01E-15 | 1.34 |
| CXCR3 | 4.30E-14 | 8.16E-13 | 1.33 |
| GBP1 | 4.34E-10 | 2.74E-09 | 1.33 |
| LY9 | 9.97E-14 | 1.57E-12 | 1.33 |
| ICOS | 1.57E-13 | 2.24E-12 | 1.33 |
| IRF8 | 2.45E-17 | 3.16E-15 | 1.32 |
| LILRB4 | 8.42E-10 | 5.03E-09 | 1.31 |
| CD8B | 3.08E-13 | 3.97E-12 | 1.31 |
| PRF1 | 3.10E-10 | 2.04E-09 | 1.28 |
| FASLG | 3.91E-10 | 2.52E-09 | 1.27 |
| GZMB | 4.45E-08 | 1.99E-07 | 1.25 |
| CD5 | 6.50E-16 | 2.33E-14 | 1.24 |
| CD45RA | 4.21E-10 | 2.69E-09 | 1.24 |
| PTPRC | 8.39E-17 | 5.08E-15 | 1.22 |
| CD274 | 2.24E-11 | 1.83E-10 | 1.22 |
| CD40LG | 1.04E-10 | 7.92E-10 | 1.19 |
| GZMA | 1.97E-08 | 9.41E-08 | 1.19 |
| CD38 | 5.33E-09 | 2.73E-08 | 1.18 |
| CD3G | 2.05E-11 | 1.69E-10 | 1.17 |
| TNFRSF9 | 1.11E-09 | 6.17E-09 | 1.17 |
| IRF1 | 1.39E-13 | 2.04E-12 | 1.16 |
| SERPINA1 | 1.04E-06 | 3.84E-06 | 1.15 |
| IL21R | 2.29E-09 | 1.22E-08 | 1.14 |
| HLA-DQA2 | 4.85E-06 | 1.65E-05 | 1.14 |
| CCR5 | 1.87E-12 | 1.91E-11 | 1.14 |
| IL32 | 1.79E-14 | 3.99E-13 | 1.14 |
| CXCR6 | 4.65E-11 | 3.70E-10 | 1.14 |
| TNF | 2.85E-14 | 5.57E-13 | 1.13 |
| CD45RO | 1.44E-16 | 7.05E-15 | 1.12 |
| CCL4 | 5.39E-08 | 2.35E-07 | 1.12 |
| CD28 | 1.35E-11 | 1.18E-10 | 1.12 |
| XCL1/2 | 4.73E-10 | 2.96E-09 | 1.11 |
| BTLA | 2.19E-08 | 1.02E-07 | 1.11 |
| PDCD1 | 9.44E-10 | 5.58E-09 | 1.11 |
| CXCR4 | 1.16E-11 | 1.04E-10 | 1.09 |
| CD2 | 7.17E-17 | 5.08E-15 | 1.08 |
| GBP4 | 2.65E-07 | 1.08E-06 | 1.08 |
| IL2RA | 2.06E-10 | 1.38E-09 | 1.07 |
| BIRC3 | 1.09E-09 | 6.09E-09 | 1.06 |
| GZMM | 1.79E-08 | 8.60E-08 | 1.06 |
| SOCS1 | 1.31E-12 | 1.41E-11 | 1.06 |
| CD80 | 1.05E-09 | 5.95E-09 | 1.05 |
| ITGA4 | 1.65E-11 | 1.40E-10 | 1.05 |
| NCR1 | 7.59E-09 | 3.80E-08 | 1.04 |
| JAK3 | 3.91E-12 | 3.71E-11 | 1.03 |
| CCL22 | 1.05E-07 | 4.39E-07 | 1.03 |
| STAT1 | 9.61E-13 | 1.11E-11 | 1.02 |
| CD244 | 1.00E-09 | 5.76E-09 | 1 |

**Supplementary Table S5**. The 3 differentially expressed genes between non-brisk TILs and absent TILs (T-test: fold change >2, q<0.05)

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene\_Symbol** | **p-value** | **q-value** | **Difference (log2 FC)** |
| CXCL9 | 2.11E-05 | 0.000239617 | 1.40 |
| CXCL10 | 5.35E-05 | 0.000505257 | 1.23 |
| CD27 | 1.70E-08 | 3.88E-06 | 1.02 |

**Supplementary Table S6**. The 31 differentially expressed genes between non-brisk+ brisk TILs and absent TILs (T-test: fold change >2. q<0.05)

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene\_Symbol** | **p-value** | **q-value** | **Difference (log2 FC)** |
| CD247 | 1.32E-10 | 1.09E-09 | 1.00 |
| LTB | 1.74E-11 | 1.66E-10 | 1.00 |
| CTLA4 | 4.83E-12 | 5.79E-11 | 1.01 |
| IL2RB | 1.29E-13 | 2.86E-12 | 1.03 |
| CCL19 | 2.61E-12 | 3.56E-11 | 1.05 |
| CCL5 | 8.21E-11 | 7.13E-10 | 1.06 |
| SLAMF7 | 8.78E-12 | 9.87E-11 | 1.06 |
| CTSW | 4.85E-12 | 5.79E-11 | 1.07 |
| GNLY | 5.97E-08 | 2.92E-07 | 1.08 |
| CD96 | 1.55E-14 | 7.42E-13 | 1.08 |
| IL7R | 1.94E-12 | 2.85E-11 | 1.08 |
| TIGIT | 1.50E-11 | 1.59E-10 | 1.08 |
| CD3E | 2.33E-14 | 7.42E-13 | 1.09 |
| TRAT1 | 1.35E-13 | 2.86E-12 | 1.10 |
| SPIB | 1.60E-11 | 1.61E-10 | 1.10 |
| CD79A | 5.37E-05 | 0.000162892 | 1.10 |
| CD6 | 7.58E-13 | 1.28E-11 | 1.11 |
| CD3D | 2.14E-14 | 7.42E-13 | 1.11 |
| ITGAL | 1.19E-15 | 2.27E-13 | 1.11 |
| LCK | 2.22E-14 | 7.42E-13 | 1.13 |
| CD8A | 2.01E-11 | 1.83E-10 | 1.13 |
| CXCL11 | 4.08E-06 | 1.47E-05 | 1.16 |
| CXCL13 | 7.36E-06 | 2.51E-05 | 1.20 |
| NKG7 | 2.05E-10 | 1.45E-09 | 1.21 |
| SH2D1A | 8.01E-13 | 1.28E-11 | 1.22 |
| GZMK | 2.16E-13 | 4.13E-12 | 1.23 |
| KLRK1 | 2.74E-14 | 7.48E-13 | 1.26 |
| IDO1 | 9.95E-10 | 6.13E-09 | 1.26 |
| CD27 | 1.33E-14 | 7.42E-13 | 1.33 |
| CXCL10 | 9.10E-09 | 5.11E-08 | 1.69 |
| CXCL9 | 2.48E-10 | 1.63E-09 | 1.962 |